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Landscape Position Influences Microbial Composition and Function via Redistribution of Soil Water across a Watershed

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Subalpine forest ecosystems influence global carbon cycling. However, little is known about the compositions of their soil microbial communities and how these may vary with soil environmental conditions. The goal of this study was to characterize the soil microbial communities in a subalpine forest watershed in central Montana (Stringer Creek Watershed within the Tenderfoot Creek Experimental Forest) and to investigate their relationships with environmental conditions and soil carbonaceous gases. As assessed by tagged Illumina sequencing of the 16S rRNA gene, community composition and structure differed significantly among three landscape positions: high upland zones (HUZ), low upland zones (LUZ), and riparian zones (RZ). Soil depth effects on phylogenetic diversity and β-diversity varied across landscape positions, being more evident in RZ than in HUZ. Mantel tests revealed significant correlations between microbial community assembly patterns and the soil environmental factors tested (water content, temperature, oxygen, and pH) and soil carbonaceous gases (carbon dioxide concentration and efflux and methane concentration). With one exception, methanogens were detected only in RZ soils. In contrast, methanotrophs were detected in all three landscape positions. Type I methanotrophs dominated RZ soils, while type II methanotrophs dominated LUZ and HUZ soils. The relative abundances of methanotroph populations correlated positively with soil water content ($R = 0.72$, $P < 0.001$) and negatively with soil oxygen ($R = -0.53$, $P = 0.008$). Our results suggest the coherence of soil microbial communities within and differences in communities between landscape positions in a subalpine forested watershed that reflect historical and contemporary environmental conditions.

In the western United States, approximately 70% of carbon sink activity is located at elevations above 750 m, where 50 to 85% of land is dominated by hilly or mountainous topography (1). Fluxes of carbonaceous gases, such as carbon dioxide (CO_2) and methane (CH_4), significantly affect the size of the carbon sink, with soil respiration accounting for the largest terrestrial CO_2 flux to the atmosphere (2). CO_2 in soil pore spaces is derived primarily from autotrophic (root) and heterotrophic (microbe) respiration, which is mediated by environmental factors such as temperature, soil water content (SWC), O_2 availability, and organic matter (3–5). The direction and intensity of CH_4 flux depends on the local balance of the CH_4 consumption by methanotrophs and CH_4 production by methanogens, both of which also are subject to such environmental influences. Because diffusive gas transport through soils is reduced with increasing SWC, hydrologic variations can strongly affect soil O_2 levels, which in turn influence the relative rates of (anaerobic) methanogenesis and (aerobic) methanotrophy. Although saturated soils (e.g., wetlands) are major terrestrial sources of CH_4 emissions (6), emission may at times occur from unsaturated soils, depending on the fine-scale heterogeneity of soil redox status (7); in some cases, CH_4 source/sink switching behavior is observed with seasonal flooding or drydown (8–12).

Little is known about how soil microbial community structure is influenced by both historical and contemporary environmental conditions of subalpine forested soils (13) and how microbial community structure might correlate with soil fluxes of CO_2 and CH_4. Landscape factors that may influence the occurrence and abundance of microorganisms include geographic location (14), topographic features such as drainages (15), and soil characteristics across spatial scales (16). Contemporary soil environmental conditions include organic C availability (17), nutrient content (18), SWC and temperature (19), and vegetative cover (20). Forested subalpine watersheds often are heterogeneous with respect to both historical and contemporary environmental conditions. To date, a watershed-wide assessment of the variability of soil microbial communities within the context of environmental conditions imposed by landscape heterogeneity is lacking.

Over the past decade, research efforts at the Tenderfoot Creek Experimental Forest (TCEF) (Fig. 1) within the Lewis and Clark National Forest, Montana, have focused on the spatial and tem-

Received 14 August 2015 **Accepted** 28 September 2015

Accepted manuscript posted online 2 October 2015

Citation Du Z, Riveros-Iregui DA, Jones RT, McDermott TR, Dore JE, McGlynn BL, Emanuel RE, Li X. 2015. Landscape position influences microbial composition and function via redistribution of soil water across a watershed. Appl Environ Microbiol 81:8457–8468. doi:10.1128/AEM.02643-15.

Editor: F. E. Löffler

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.02643-15.

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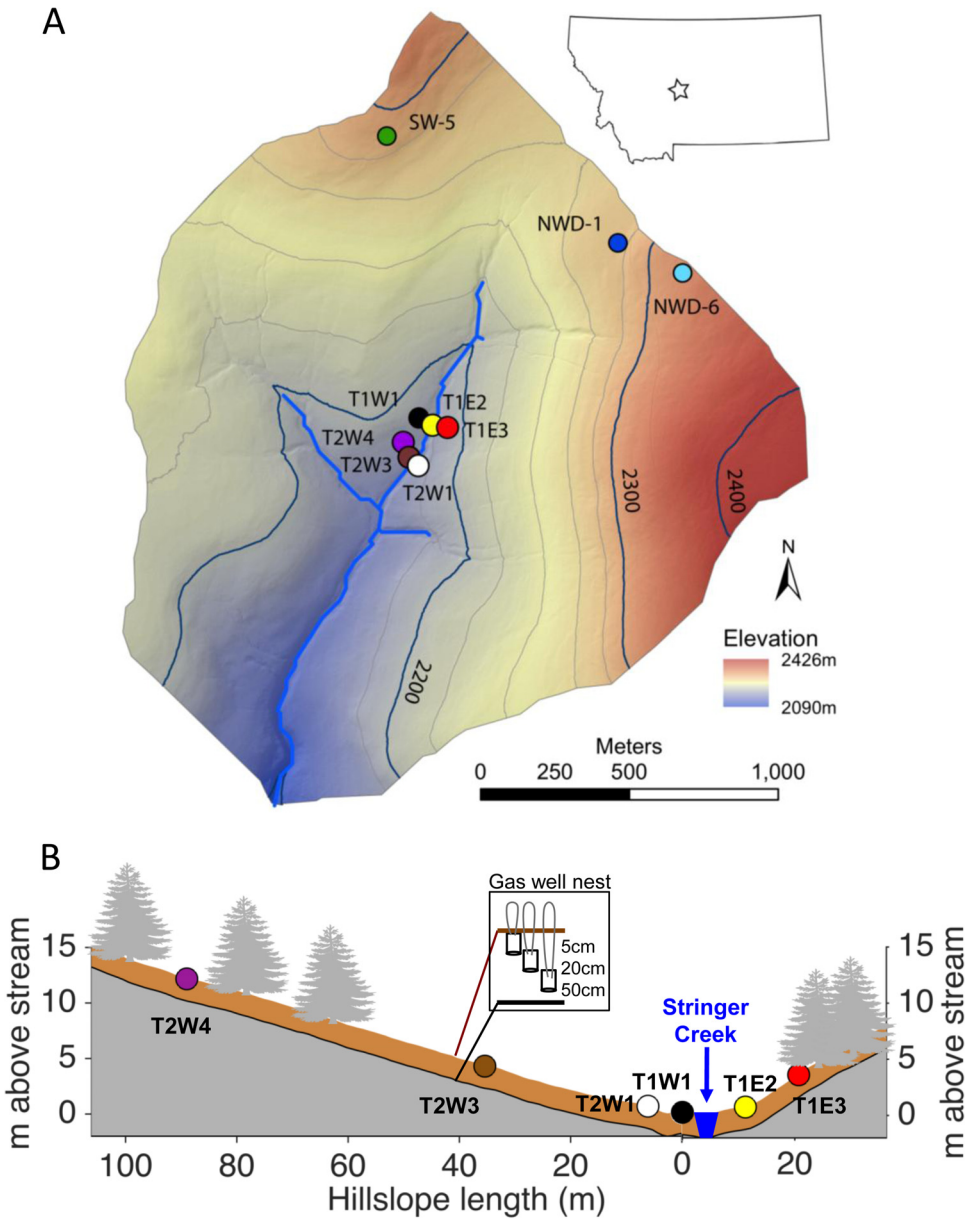

FIG 1 Stringer Creek research site located within the Tenderfoot Creek Experimental Forest, Montana. (A) Shaded relief of elevation within the Stringer Creek Watershed. Sampling sites examined in this study illustrated as color-coded dots (to match the sites shown in Fig. 3). (B) Cross-section (note vertically exaggerated soil and elevation) depicting two of the transects illustrating the sampling sites relative to the creek and their topographic positions. Each sampling site was located adjacent to a previously installed gas well nest set at 5 cm, 20 cm, and 50 cm (inset shown accompanying T2W3) that allowed for the sampling of soil O_2, CO_2, and CH_4.

poral scaling of hydrological, biogeochemical, and ecological processes across the larger Tenderfoot Creek Watershed, with particular focus on the Stringer Creek drainage. These studies have included watershed hydrology (e.g., stream water sources, flow paths, and riparian dynamics) (21, 22), relationships between hydrologic conditions and CO_2 efflux across landscape positions (23, 24), and landscape-scale land-atmosphere CO_2, H_2O, and energy fluxes (25, 26). This site is characteristic of vast extents of forests in the northern Rocky Mountains and continues to be the focus of studies aimed at generating models that accurately describe and explain the biotic and abiotic processes that contribute to subalpine ecosystem function.

In this study, we investigated soil microbial community structure and function across the Upper Stringer Creek Watershed in relation to the variability of major topographical features, environmental factors, and soil gas composition. Specifically, the objectives of this study were to (i) characterize and compare the microbial communities in drier upland soils and wetter riparian meadows and (ii) investigate the potential relationships among *Bacteria* and *Archaea*, environmental factors, and soil gas measurements. Data to address these objectives included soil CO_2 efflux, CO_2 concentration, and CH_4 concentration, as well as the SWC, temperature, pH, and O_2 content of soils.

MATERIALS AND METHODS

Site description and sample collection. TCEF is located in the Little Belt Mountains of central Montana (46°55′ N; 110°54′ W). It is a subalpine forest of the northern Rocky Mountains, which are believed to contribute significantly to the North American carbon sink (1). Mean annual precipitation at the site is 880 mm, with 70% falling as snow. The site is subject to a steady seasonal drydown in SWC following snowmelt (27). The mean annual temperature is 0°C, and the growing season typically lasts from early June to the end of August. The watershed land cover is largely composed of upland forests, interspersed with riparian meadows. Vegetation in riparian meadows consists primarily of *Calamagrostis canadensis* (bluejoint reedgrass), whereas upland forests consist primarily of *Pinus contorta* (lodgepole pine) and, to a lesser extent, *Abies lasiocarpa* (Subalpine fir) and *Picea engelmannii* (Engelmann Spruce). *Vaccinium scoparium* (Whortleberry) is the predominant upland understory species (28). The geology is characterized by granite gneiss, shales, quartz porphyry, and quartzite (29). The hillslopes are composed mainly of loamy-skeletal, mixed Typic Cryochrepts, whereas the riparian zones are composed of highly organic clayey, mixed Aquic Cryoboralfs (30).

Three years (2005 to 2007) of measurements of soil CO_2 efflux, soil temperature, and SWC were collected previously at 62 sites within the Stringer Creek Watershed (24, 31, 32). These prior studies established selection criteria for the nine sites that were included in this study and are referred to as NWD1, NWD6, SW5, T1E2, T1E3, T1W1, T2W1, T2W3, and T2W4 (Fig. 1A). These sites were selected on the basis of terrain analysis and site assessment and are characteristic of the different soils, slope, aspect, topographic positions, and hydrologic regimes of the watershed (26, 31). Based on hillslope positions, sites NWD1, NWD6, SW5, and T2W4 were defined as high upland zone (HUZ), sites T1E2, T1E3, and T2W3 as low upland zone (LUZ), and sites T1W1 and T2W1 as riparian zone (RZ) (Fig. 1). Soil samples at each site were collected on 10 July 2012 at three soil depths (5, 20, and 50 cm) from hand-dug pits (~50 cm in diameter). At each depth, two soil subsamples were scraped from the wall of the pit into sterile 50-ml centrifuge tubes. All soil samples were transferred to the laboratory on dry ice and stored at −80°C until analysis.

Soil environmental measurements. Soil environmental measurements were conducted at all nine sites between 8 and 11 July 2012 (see Table S1 in the supplemental material). Volumetric SWC was measured using a portable time domain reflectometry meter (Hydrosense; Campbell Scientific, Logan, UT) that reports the volumetric soil water content of the upper 12 cm of soil at each location. Soil temperature in the top 12 cm was measured using a 12-cm soil thermometer (Reotemp Instruments, San Diego, CA). Soil temperature and volumetric SWC data are presented here as means of triplicate measurements made within two meters of gas wells and soil sampling locations.

Soil gases were collected from nested gas wells previously augered and installed at three depths (5, 20, and 50 cm) and left in place since 2005 (24). A hand-held infrared CO_2 analyzer with an integral air pump (0 to 5% CO_2 working range; GM-70; Vaisala, Woburn, MA) was connected to two sampling ports on each gas well. The air from the well was circulated through the instrument and returned, creating a closed loop and minimizing pressure changes during sampling. Factory calibration of the GM-70 was validated in the laboratory using air-CO_2 mixtures. Soil O_2 concentrations were measured using a galvanic oxygen sensor (MO 200; Apogee Instruments, Logan, UT) plumbed in line within the closed sampling loop; the MO 200 was field calibrated to ambient air assumed to contain 20.95% O_2. Using a syringe, ~50 ml of soil gas was extracted from the circulation loop through a septum tee fitting and injected into a 180-ml laminated foil gas sampling bag (FlexFoil, SKC Inc., Eighty Four, PA). These bags were returned to Montana State University for analysis of CH_4 by gas chromatography with flame ionization detection. Certified CH_4 mixtures (Scotty; Air Liquide America, Houston, TX) were used to calibrate the gas chromatograph.

Surface soil CO_2 efflux was measured using a portable infrared gas analyzer (EGM-4; accuracy within 1% of calibrated range [0 to 2,000 ppm]; PP Systems, MA) connected to a soil respiration chamber (SRC-1; footprint, 78 cm^2; PP Systems). All CO_2 efflux measurements are reported as means from triplicate measurements made on undisturbed ground within two meters of the gas well nests. Additional details on the soil CO_2 efflux measurements were reported by Pacific et al. (24).

DNA extraction and sequencing. DNA was extracted from 1-g soil subsamples using the FastDNA SPIN kit for soil (MP BIO Biomedicals, Santa Ana, CA) by following the manufacturer's instructions. DNA extracts were purified using a desalting procedure and using the OneStep PCR inhibitor removal kit (Zymo Research Corporation, Irvine, CA). Purified DNA extracts were quantified using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA) and PCR tested prior to submission for sequencing. DNA extracts then were overnight express shipped to the Institute for Genomics & Systems Biology Next Generation Sequencing Core at Argonne National Laboratory for PCR amplification using primers 515F and 806R targeting the V4 region of the 16S rRNA gene in the domains *Bacteria* and *Archaea* (33). Amplicons were sequenced using the Illumina MiSeq sequencing platform.

The 16S rRNA gene was used as a molecular marker for estimating the relative abundance of methanotrophs (34), because the 16S rRNA gene and functional gene *pmoA* cover nearly identical similarities for methanotrophic populations in environmental samples (35), although we note that it does not track the forest soil methanotroph that so far is known only by its *pmoA* sequence (36). The following genera were considered methanotrophic bacteria in this work based on previous studies (37, 38): *Methylobacter*, *Methylomicrobium*, *Methylomonas*, *Methylocaldum*, *Methylococcus*, *Methylosoma*, *Methylosarcina*, *Methylothermus*, *Crenothrix*, *Clonothrix*, *Methylosphaera*, *Methylocapsa*, *Methylocella*, *Methylosinus*, and *Methylocystis*.

Sequencing and analysis. A total of 3.14 gigabytes of sequence was generated and later processed using Quantitative Insights Into Microbial Ecology (QIIME), version 1.7.0 (39). Chimera sequences were identified and removed using USEARCH 6.1 (40), which detected chimeras using reference operational taxonomic units (OTUs) in Greengenes defined at 97% identity and performed *de novo* chimera detection based on the abundances of input sequences (41). Low-quality sequences were removed using the default filter parameters in QIIME: quality score of <25, minimum and maximum lengths of 200 and 1,000, respectively, maximum number of homopolymer runs ($n = 6$), no ambiguous bases allowed, and no mismatches allowed in the primer sequence.

Phylotypes were determined with UCLUST at a default sequence similarity level of 97% (96). The representative sequences for each phylotype were aligned against the Greengenes core set using PyNAST (42). The sequences then were classified using the BLAST taxonomy assignment (43). Alignments were filtered to remove uninformative data and sequence gaps using the Greengenes alignment Lane mask file (44), and subsequently phylogenetic trees were built with FastTree (45). Based on the OTU summary, sequence libraries containing fewer than 10,000 sequences were considered low quality and were excluded from further analyses. The smallest library included in this study contained 17,699 sequences. OTU tables were rarefied to a sampling depth of 15,000 sequences per library. Alpha diversity (diversity of microbial communities found within individual samples) was estimated with rarefied OTU tables using Faith's phylogenetic diversity (PD) metric (46), Shannon index, Chao1 index, and observed species. Beta diversity (diversity of microbial communities found between different samples) was estimated with rarefied OTU tables by weighted-UniFrac distances (47).

Statistical analysis. Sequence libraries from duplicate DNA extracts (i.e., technical replicates) were merged prior to the Mantel tests and ADONIS analyses. Mantel tests were conducted in QIIME to test the significance of correlations between weighted UniFrac distances of soil microbial communities and the normalized Euclidean distances in environmental factors and soil carbonaceous gas measurements. Pearson correlations were performed using the software R (R Foundation

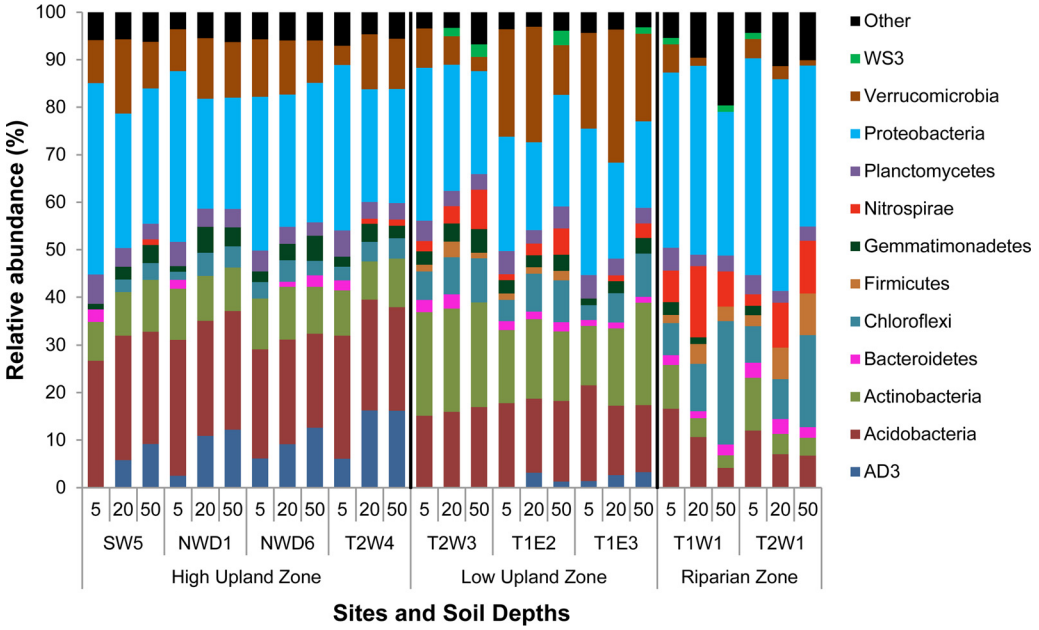

FIG 2 Relative abundance of dominant bacterial phyla at the three soil depths (5 cm, 20 cm, and 50 cm) at the nine sites sampled within the Stringer Creek Watershed.

for Statistical Computing, Vienna, Austria) to identify correlations between relative abundances of major bacterial phyla or methanotrophs as a function of environmental factors and carbonaceous gas measurements.

Nucleotide sequence accession number. The sequences determined in this work deposited in the NCBI Sequence Read Archive (SRA) under the accession number SRP052862.

RESULTS

Soil environmental measurements. *In situ* soil environmental measurements were conducted within a maximum of 1 to 2 days before or after soil samples were collected for microbial analyses. Volumetric SWC ranged from 6.0% to 12.4% in the HUZ soils, from 6.3% to 49.4% in LUZ sites, and from 48.9% to saturation in RZ soil profiles (see Table S1 in the supplemental material). Soil temperature ranged from 11.2 to 15.9°C across all sites. At HUZ and LUZ sites, most of the soil O_2 levels were within a narrow range (20.2 to 21.4%) near that of the atmosphere (20.95%) and varied little across depths (see Table S1). In contrast, soil O_2 declined with depth at the RZ sites (see Table S1). Soil pH ranged from 4.22 to 5.64 in HUZ sites, from 5.63 to 6.86 in LUZ sites, and from 5.41 to 6.30 in RZ sites. Soil CO_2 and CH_4 concentrations and surface CO_2 efflux were consistently higher in RZ sites than in LUZ and HUZ sites (see Table S1), in agreement with previous reports on CO_2 from this site (23, 24, 26, 31).

General analyses of the sequencing libraries. Four DNA extracts (duplicate DNA extractions for each of the two soil subsamples for each depth) were used to establish four sequence libraries for each of the 27 soil samples (9 sites times 3 soil depths). Nine of the 108 DNA extracts failed to yield quality libraries; these included one extract each from T1E2 5 cm, T1E2 20 cm, T1W1 5 cm, T2W1 5 cm, T2W1 20 cm, T2W1 50 cm, and T2W3 5 cm and two extracts from NWD1 5 cm. The remaining 99 libraries consisted of a total of 5,572,763 sequences, ranging from 17,699 to 155,603 sequence reads per library, and were rarefied to 15,000 reads each. Rarefaction curves (see Fig. S1 in the supplemental material) suggested the sequencing effort recovered the dominant taxa at a genetic distance of 3%. At RZ sites, the OTU counts at 5 cm were higher than those at 20 cm ($P = 0.009$) and at 50 cm ($P = 0.003$). In comparison, the trends at HUZ and LUZ sites were less pronounced. With two replicate libraries for each soil subsample, analysis of similarity (ANOSIM) tests showed that the two subsamples were similar ($P > 0.05$ for all soil samples tested). Consequently, diversity and richness assessments were conducted based on the average of replicate libraries.

Taxonomic diversity. Microbial community composition varied between the three landscape positions in the watershed, i.e., HUZ, LUZ, and RZ. *Bacteria* were more abundant than *Archaea* in all libraries (see Table S2 in the supplemental material). A total of 25 bacterial phyla were identified across the entire sample set; some phyla were undetectable in some soils/depths (see Table S3). In all sequence libraries, *Proteobacteria* (18.15% to 45.59%), *Acidobacteria* (3.92% to 28.62%), *Verrucomicrobia* (0.94% to 27.93%), and *Actinobacteria* (2.51% to 21.69%) were dominant (Fig. 2; also see Table S3). Other phyla that were consistently detected included *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, *Nitrospirae*, and *Planctomycetes*. Rare phyla, defined as those with a relative abundance of less than 1%, were clustered together in the "other" category (Fig. 2). Because methanotrophs are a functional group of interest in this study and belong to *Alphaproteobacteria* and *Gammaproteobacteria*, these two subphyla were examined in greater detail (see Fig. S2). Both subphyla, particularly the *Alphaproteobacteria*, were most abundant at the 5-cm depth and typically declined with depth (see Fig. S2).

Archaea made up small portions of the communities, ranging in relative abundance from undetectable to 4.85% (see Table S2 in the supplemental material). In most locations, they were more abundant at the 20- and 50-cm depths than at 5 cm and were lowest in HUZ sites and highest in RZ sites (see Table

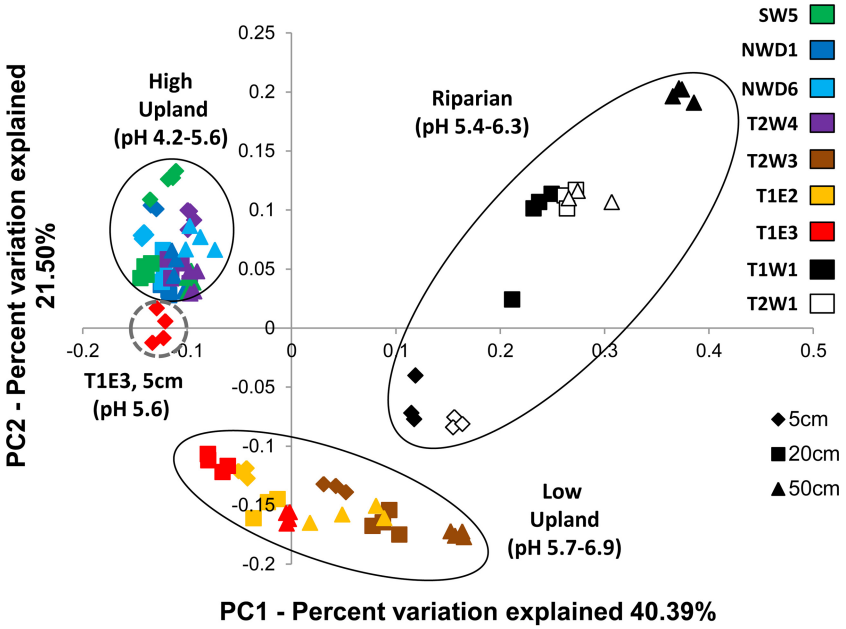

FIG 3 Principal coordinate analysis of β-diversity observed in the Stringer Creek soil microbial communities. Grouping of the sampling sites into high upland (referred to as HUZ in the text), low upland (LUZ), and riparian (RZ) zones are shown by black circles and are supported by ADONIS analysis (see Table S6 in the supplemental material). The T1E3 5-cm-depth community (red diamonds) is distinguished by the gray dashed circle because its composition appears transitional between the HUZ and LUZ communities (see Table S6).

S2). *Crenarchaeota* and *Euryarchaeota* were the dominant phyla (see Table S4), with *Crenarchaeota* dominating in all locations except the two RZ sites (T1W1 and T2W1). The relative abundance of these phyla was similar at T1W1, while the *Euryarchaeota* were more abundant than *Crenarchaeota* at T2W1 (see Table S4).

Shannon and Chao1 indices were calculated to estimate and compare the microbial richness and diversity at different depths and locations (see Table S5 in the supplemental material). In general, α diversity did not pattern with depth in the HUZ soils, whereas it decreased with soil depth in the LUZ and RZ soils (see Table S5 and Fig. S3). Additionally, the communities in the HUZ soils exhibited lower diversity than those in the LUZ and RZ (see Fig. S3).

Principal coordinate analysis (PCoA) then was employed to examine the relative relatedness of the various microbial communities (Fig. 3). The two coordinates accounted for 40.39% and 21.50% of the total variation, respectively. The microbial communities could be distinguished in a manner that clearly related community structure with landscape positions. For the most part, replicate libraries clustered closely, consistent with the above-mentioned ANOSIM analysis. In general, additional ADONIS comparisons were largely consistent with the PCoA clustering, primarily delineating communities to within the HUZ, LUZ, and RZ landscape positions ($R^2 = 0.3379$ to 0.4752; $P < 0.001$) (see Table S6 in the supplemental material) as illustrated in Fig. 3. One location of particular interest was T1E3, which represents a transition zone between the HUZ and LUZ landscape positions (Fig. 1B). More specifically, the T1E3 5-cm libraries clustered distinctly away from the other, deeper T1E3 communities (20 cm and 50 cm) (Fig. 3). ADONIS analysis of the T1E3 5-cm community agreed with its PCoA separation from the T1E3 20-cm and 50-cm communities, although the distinction was only marginally significant ($P = 0.066$) (see Table S6) and implied relatively weak similarity to either the HUZ or LUZ group (see Table S6).

Correlation with environmental factors and soil carbonaceous gases. In order to better understand the community composition and diversity patterns (Fig. 2 and 3), Mantel tests were conducted to examine the relationships between community composition and soil environmental measurements (Table 1). Statistically significant (all P values <0.001) correlations of various strengths were observed for SWC, soil O_2, and soil pH (Table 1). Soil CO_2 efflux, CO_2 concentration, and CH_4 concentration also correlated with community structure (Table 1). Phylogenetic diversity, a measure of alpha diversity, was positively correlated with both SWC and soil CO_2 efflux (Fig. 4A and B). SWC and soil CO_2 efflux also exhibited a strong positive correlation (Fig. 4C). Pearson correlation analysis was conducted to individually examine the relative abundance of major bacterial phyla relative to en-

TABLE 1 Mantel correlations relating bacterial community composition, environmental factors, and soil carbonaceous gas measurements

Parameter	Mantel correlation	*P* value
Environmental factors		
SWC (%)	0.68	<0.001
Soil temp (°C)	0.18	0.002
Soil O_2 (%)	0.42	<0.001
Soil pH	0.34	<0.001
Soil carbonaceous gases		
CO_2 efflux (g m^{-2} h^{-1})	0.33	<0.001
CO_2 (ppm)	0.45	<0.001
CH_4 (ppm)	0.32	<0.001

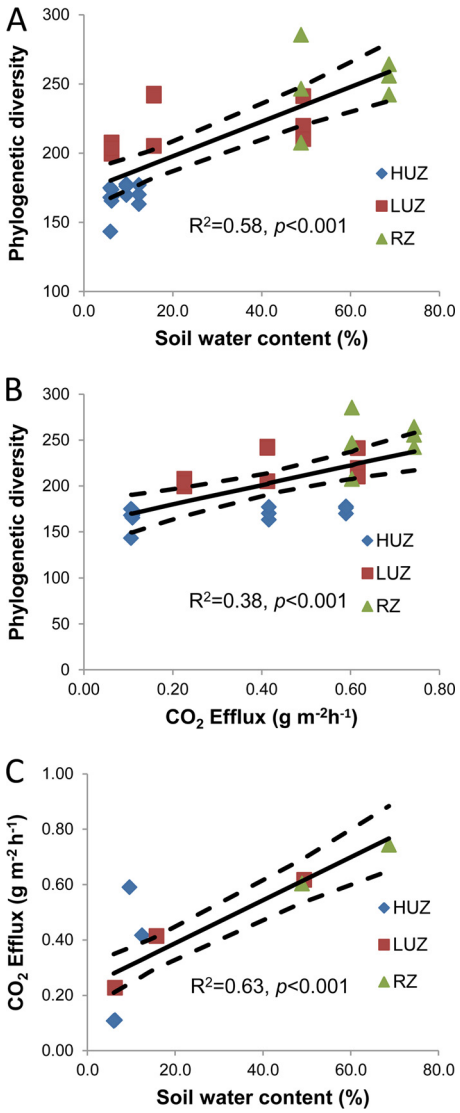

FIG 4 Soil water content exhibited positive correlation with both microbial phylogenetic diversity (A) and CO_2 effluxes (B). (C) Correlation between the phylogenetic diversity and CO_2 effluxes. Solid lines are linear regression lines, while the dashed lines illustrate the 95% confidence intervals. Phylogenetic diversity was measured for each soil depth, while CO_2 flux was measured for each site, yielding only 9 data points. SWC, CO_2 efflux, and PD values for two HUZ sites, SW5 and NWD6, are very similar, explaining the early overlap.

vironmental factors and soil carbonaceous gas measurements (Table 2). Correlations varied in direction, strength, and pattern. For example, the relative abundance of *Chloroflexi* was positively correlated with SWC, pH, and all soil carbonaceous gas measurements but was negatively correlated with soil O_2 (Table 2). In contrast, the relative abundance of *Acidobacteria* was positively correlated with soil O_2 and negatively correlated with SWC, pH, CO_2 efflux, and CO_2 concentrations (Table 2).

Methane cycling microbes. With the exception of the 20-cm sample at NWD6 (6 of 15,000 reads), methanogens were detected only in soils from RZ sites (Fig. 5A). At the genus level, *Methanobacterium*, *Methanosaeta*, and *Methanosarcina* were detected in all riparian libraries, whereas *Methanocella* and *Methanospirillum* were less abundant and detected only in the deeper RZ soil horizons (Fig. 5B). The 16S rRNA signatures of various known methanotroph genera were detected in 21 of the 27 soil samples (Fig. 6A). RZ sites T1W1 and T2W1 had the highest relative abundance (up to ~0.96%) of methanotrophs (Fig. 6A). Type II methanotrophs (annotated to the genera *Methylosinus* and *Methylocella* of the *Alphaproteobacteria*) dominated in the HUZ topographies, whereas type I methanotrophs (*Methylomonas*, *Methylocaldum*, and *Crenothrix* of the *Gammaproteobacteria*) were most prevalent in the RZ soils (Fig. 6A and B). *Crenothrix* was the most abundant methanotroph (0.07% to ~0.72%), whereas *Methylomonas* and *Methylocella* were the least abundant (<0.01%). Relationships between the relative abundances of methanotrophs and environmental factors and soil carbonaceous gases also were examined using Pearson correlation (Table 3). The relative abundance of methanotrophs (especially type I) was positively correlated with SWC ($R = 0.72$; $P < 0.001$). Both types of methanotrophs were negatively correlated with soil O_2 levels (particularly type II). All soil carbonaceous gas measurements exhibited statistically significant positive correlations with methanotroph relative abundance (Table 3).

DISCUSSION

Given the extensive distribution of subalpine forests, a better global understanding of how these ecosystems contribute to C exchange with the atmosphere is critical (1, 48). Surprisingly, there is little information regarding the soil microbial communities involved. This experimental forest has been studied extensively in efforts to quantify soil CO_2 production and surface efflux as a function of hydrology at the landscape scale (23, 24, 26, 31, 32, 49, 50). The current study aimed to continue these landscape-scale efforts by assessing potential linkages between different soil environments within this watershed and the microbial drivers of greenhouse gas exchanges. The nine sampling sites were selected based on prior research that had identified landscape positions in this drainage that differed with respect to soil environmental variables and gas fluxes (26, 31). This sampling strategy allowed us to identify how community structural patterns differed among landscape positions and how they might be correlated with key soil environmental factors (e.g., SWC, temperature, O_2, and pH) and ecosystem function (carbonaceous gas fluxes/concentrations).

Landscape position in this watershed was important in shaping microbial communities (Fig. 3), with differences being observed at the phylum level (Fig. 2). Riparian zones and upland zones often exhibit different rates of microbially mediated soil processes due to the distinct soil moisture regimes (51) and differing microbial community compositions (52). The distinct microbial community structural patterns revealed in this study suggest that deterministic processes associated with habitat specialization are important. Snowmelt events offer significant annually repeated opportunities for the downslope redistribution of microbes from HUZ to LUZ or to RZ positions, yet distinct community structure and diversity patterns were evident (Fig. 2 and 3 and Table 2). There likely are several contributing factors. Soil temperature appeared to have little effect on most phyla, likely due to the narrow range at the time of sampling (11.2 to 15.9°C; see Table S1 in the supplemental material). However, SWC stands out as a major deterministic selector. Strong correlations of SWC with community structure were shown in both Mantel tests (Table 1; $R = 0.68$) and canonical analysis of principal coordinates (see Fig. S4). This is consistent with prior investigations demonstrating similar SWC

TABLE 2 Pearson correlation analysis of environmental parameters with main phyla of all soil samples[a]

Phylum	SWC (%)		Soil T (°C)		Soil O_2 (%)		Soil pH		CO_2 efflux (g $m^{-2}h^{-1}$)		CO_2 (ppm)		CH_4 (ppm)	
	R	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value
AD3	-0.59	0.001	0.01	0.974	0.30	0.155	-0.41	0.036	-0.29	0.149	-0.31	0.141	-0.21	0.325
Acidobacteria	-0.76	<0.001	0.21	0.293	0.47	0.022	-0.70	<0.001	-0.55	0.003	-0.54	0.006	-0.36	0.088
Actinobacteria	-0.13	0.523	0.46	0.015	0.10	0.635	0.33	0.093	-0.11	0.590	-0.05	0.810	-0.36	0.081
Bacteroidetes	0.55	0.003	-0.12	0.535	-0.29	0.165	0.12	0.564	0.38	0.052	0.30	0.154	0.03	0.903
Chloroflexi	0.62	<0.001	-0.08	0.710	-0.49	0.014	0.61	<0.001	0.50	0.008	0.59	0.003	0.41	0.048
Firmicutes	0.82	<0.001	-0.17	0.399	-0.70	<0.001	0.41	0.035	0.64	<0.001	0.74	<0.001	0.44	0.033
Gemmatimonadetes	-0.39	0.042	0.38	0.048	0.17	0.416	0.06	0.759	-0.29	0.144	-0.17	0.432	-0.26	0.228
Nitrospirae	0.76	<0.001	-0.01	0.973	-0.71	<0.001	0.60	<0.001	0.60	<0.001	0.76	<0.001	0.77	<0.001
Planctomycetes	-0.34	0.082	0.08	0.705	0.49	0.015	-0.46	0.017	-0.22	0.275	-0.54	0.006	-0.35	0.093
Proteobacteria	0.52	0.006	-0.22	0.270	-0.27	0.196	-0.36	0.067	0.29	0.136	0.22	0.305	0.30	0.158
Verrucomicrobia	-0.66	<0.001	-0.25	0.213	0.44	0.031	0.03	0.875	-0.56	0.002	-0.41	0.047	-0.33	0.120
WS3	0.33	0.091	0.26	0.189	-0.15	0.486	0.57	0.002	0.28	0.164	0.21	0.320	-0.10	0.646

[a] Dark gray shaded entries highlight statistically significant ($P < 0.05$) positive correlations, whereas light gray shaded entries denote significant negative correlations.

relationships with microbial biomass and soil respiration (53–55). The relative abundance of a particular microorganism often is influenced in real time by the prevailing moisture in the soil pore environment (a contemporary environmental factor). Topography can significantly influence water movement; thus, it can influence relationships between landscape position and microorganisms (Fig. 2, 3, and 4). Major bacterial phylum differences between upland and riparian zones most noticeably involved *Aci-*

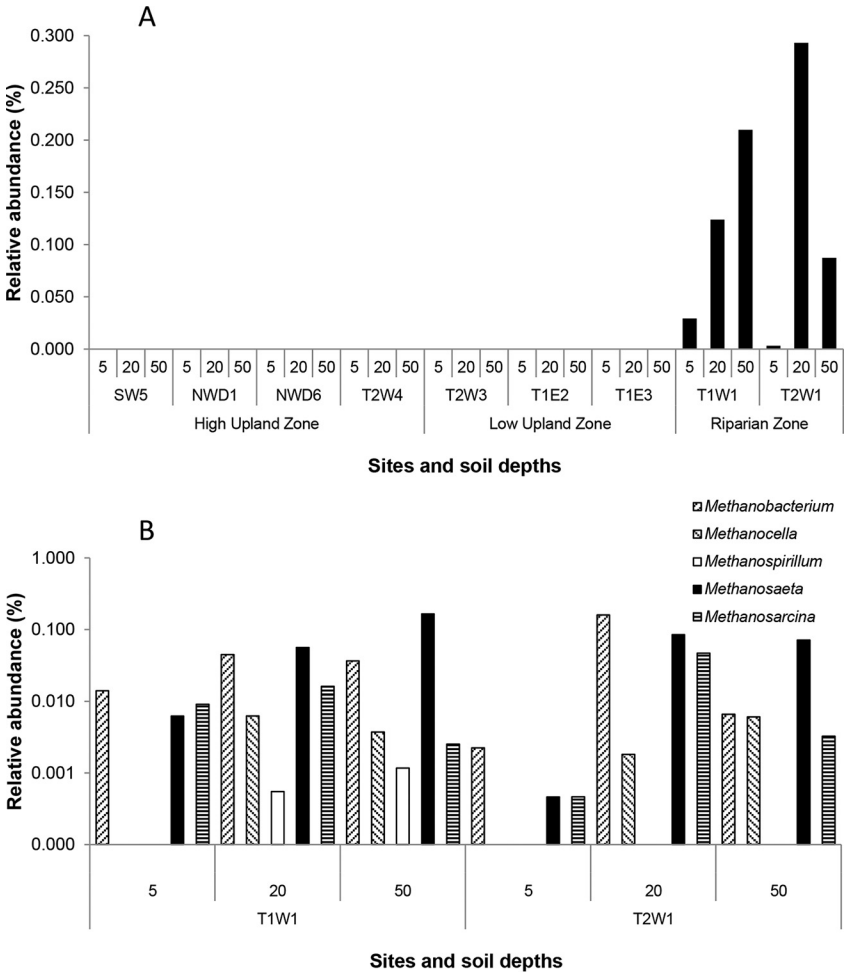

FIG 5 Relative abundance of total methanogens in soil microbial communities (A) and the relative abundance of different methanogenic genera at the two RZ sites, T1W1 and T2W1 (B).

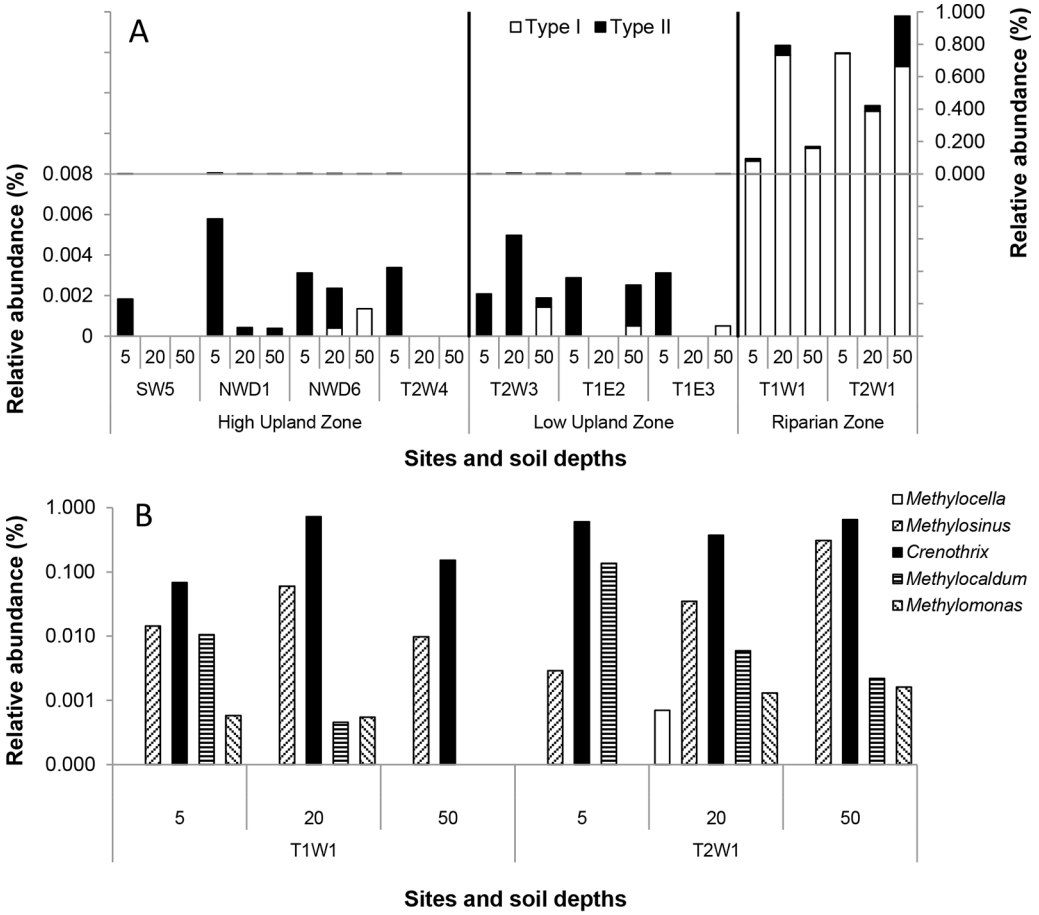

FIG 6 Relative abundance of methanotrophic bacteria in sampled soils. (A) Total methanotrophs delineated as type I (white bars) and type II (black bars). (B) Relative abundance of identified methanotroph genera in the two RZ sites, T1W1 and T2W1.

dobacteria, *Actinobacteria*, *Chloroflexi*, and *Verrucomicrobia* (Fig. 2). Considering the entire growing season in the TCEF watershed (May to August), HUZ soils are the first to dry down, and SWC in LUZ soils tends to be higher than that in HUZ soils for longer periods due to the downslope redistribution of snowmelt. Perhaps it is not surprising that the relative abundance of *Acidobacteria* and *Actinobacteria* was lower in riparian soils than in upland soils. Riparian soils generally are less aerated due to high SWC (they can be saturated much of the growing season); hence, they are not optimum for phyla, such as *Acidobacteria* and *Actinobacteria*, that include a substantial number of obligate aerobes.

Soil pH was likely another deterministic factor in this ecosystem. The pH range in the HUZ soils was largely outside that of the LUZ or RZ soils (Fig. 3; also see Table S1 in the supplemental material) and is differentially correlated with the various phyla (Table 2) so as to contribute to the clustering of HUZ communi-

TABLE 3 Pearson correlation analysis of methanotroph relative abundance as a function of environmental parameters measured in this study

Parameter	Total		Type I		Type II	
	R	*P* value	*R*	*P* value	*R*	*P* value
Environmental factors						
SWC (%)	0.72	<0.001	0.72	<0.001	0.48	0.012
Soil temp (°C)	−0.35	0.071	−0.35	0.076	−0.26	0.192
Soil O_2 (%)	−0.53	0.008	−0.51	0.011	−0.74	<0.001
Soil pH	0.22	0.263	0.2	0.317	0.24	0.229
Soil carbonaceous gases						
CO_2 efflux (g m^{-2} h^{-1})	0.54	0.004	0.54	0.004	0.36	0.069
CO_2 (ppm)	0.53	0.007	0.51	0.011	0.74	<0.001
CH_4 (ppm)	0.68	<0.001	0.65	<0.001	0.86	<0.001

ties distinct from those in the LUZ and RZ communities. Global studies of soils (56–58), as well as comparisons within the same soil profile (59), similarly have found strong connections between microbial community structure and pH.

Phylogenetic diversity also was correlated with landscape position, with the RZ and HUZ tending to represent the end members (Fig. 4; also see Fig. S3 in the supplemental material). The RZ and HUZ represent very different environments, so differences in this regard are not surprising and are consistent with previous studies, which found that soil bacterial phylogenetic diversity differed by ecosystem type (56, 59). In addition to SWC, the type and extent of vegetation also varied substantially between the riparian zone and the upland zones. As noted by Prober et al. (60), plant diversity can be a good predictor of the beta diversity of soil microbes in grassland. The full extent of this effect remains a topic for future research efforts.

The relation between ecosystem type and β-diversity reported in other studies (61, 62) also was clearly observed here (Fig. 3), although our current study is focused on a single geographical location and aimed to compare communities across landscape positions. Dispersal barriers between landscape positions can be important contributors to the β-diversity (13). Distance can in some cases act as a dispersal barrier; however, physical separation did not appear to be a factor shaping β-diversity in this drainage. Despite the significant spatial separation (up to ~1,000 m), the HUZ microbial communities were more closely related to each other than the microbial communities in the LUZ or RZ soils that were only separated by 5 to 20 m (Fig. 1 and 3). This observation is consistent with Wang and coworkers' finding that β-diversity among habitat types was significantly higher than that within habitat types (61).

Soil depth effects on microbial community structure have been observed previously in Colorado montane soils (59) and grasslands in Germany (63). In the present study, phylogenetic diversity decreased with soil depth in the riparian soils, while the trend was not as evident or consistent in the upland soils (see Fig. S3 in the supplemental material). Effects of soil depth on β-diversity and composition in the LUZ and RZ soils also were more evident than those in the HUZ soils (Fig. 3). During the July sampling dates for this study, the soil pits for both RZ sites revealed root-bound conditions at the 5-cm depth and, depending on the site, saturated conditions at the 20-cm and/or 50-cm depths. Roots were less prevalent but still conspicuous at 20 cm but were far less abundant at 50 cm. This rooting pattern, together with the SWC profile, could provide a general explanation for soil depth effects observed in the RZ soils. Though not saturated or as heavily rooted, soil horizons were apparent in the toe-slope LUZ locations. Changes in chemistry and physical properties associated with the horizonation (31) could have influenced the depth patterning observed in the LUZ soils (Fig. 3; also see Fig. S3 in the supplemental material). In a forested montane watershed, the microbial communities at various soil depths significantly differed from each other irrespective of the sampling locations within their watershed study site (59, 64). While *Bacteroidetes* and *Verrucomicrobia* were found to be the primary drivers of the distinction in microbial composition along soil profiles, no such drivers were evident in the Stringer Creek watershed. Surface soils exhibited greater β-diversity than deep soil in the montane watershed study in Colorado (59), and the organic matter composition at different soil depths was considered responsible for the vertical distinction. In contrast, the relationship between β-diversity and soil depth was not consistent across three landscape positions within the forested watershed in this study (Fig. 3).

Of the different sampling sites, the T1E3 location proved to be particularly interesting. This sampling site represents a transition point with respect to topography (changing from upland to riparian). Previous research has indicated that the hydrology and CO_2 efflux patterns of this and other Stringer Creek transition sites (21, 24, 31, 65) have characteristics of both riparian and upland zones that could affect the soil microbes. For example, saturated conditions have been observed to persist for days to weeks per year in the deeper portions of the soil profile of T1E3 (65, 66) but have not been observed in the shallow portions of the soil profile (e.g., 5 cm). β-Diversity analysis suggested the T1E3 5-cm community is more closely related to the HUZ soils than the deeper soils within the same soil profile (T1E3 20 cm and 50 cm) as well as the rest of the LUZ soils (Fig. 3). ADONIS analyses show that when the T1E3 5-cm community was included with either the HUZ or LUZ community, the resulting statistics suggested this site/depth can fit with either HUZ or LUZ soil communities (see Table S6 in the supplemental material). Difficulties in clearly assigning the T1E3 5-cm community led to it being considered a separate, transitional community, consistent with important soil selectors such as pH and moisture. The pH of the T1E3 5-cm soil (5.6) was borderline between the soils in the HUZ (4.2 to 5.6) and LUZ (5.7 to 6.9) soils.

Determining how environmental effects drive microbial function can be elusive at the phylum level because of the broad range of physiologies represented in each phylum. Growing-season soil CO_2 efflux has been shown to vary spatially across this subalpine forest landscape by as much as 7-fold (26). Correlating soil CO_2 (concentration and flux) with community composition (Table 1) and phylogenetic diversity (Fig. 4C) contributes to the ongoing discussion of the role of microbial diversity on soil respiration, a topic that has been vigorously debated and investigated (67). Studies have reported negative (68), positive (69–72), or no (68, 71, 73–76) correlations between microbial species richness and soil respiration. In this study, phylogenetic diversity exhibited a positive correlation with soil CO_2 efflux ($R^2 = 0.38$, $P < 0.001$) (Fig. 4B).

Most aspects of soil microbial heterotrophic C metabolism cannot be linked with specific phylogenetic signatures generated by Illumina sequencing. However, organisms involved in methane cycling can be distinguished at the genus level. The distribution of recognizable methanogen signatures in the Stringer Creek drainage was clear: they were below detection in all but one upland soil as opposed to comprising up to ~0.3% of the total community in the RZ soils (Fig. 5A). Identified methanogenic genera were most prevalent in the deeper RZ soil horizons (Fig. 5B), which were saturated at the time of sampling, and over the course of nearly a decade of study they have been found to generally remain so through most of the year (21, 65, 66). Therefore, the relative abundances of methanogens likely correlated with anaerobic RZ environments, which constitute only ~1.8% of the total land area in this ecosystem (21) but account for most of the CH_4 efflux (K Kaiser, B. McGlynn, and J Dore, unpublished data).

The occurrence of methanotrophs is common in the range of environments represented in this study. Recognizable type I methanotrophs were most prevalent in the RZ, while type II methanotrophs dominated in upland zones (Fig. 6A). These data strengthen and support the observations that type II metha-

notrophs dominate in mature, upland forest soils (38), whereas type I methanotrophs dominate in littoral wetland environments (77) and wet arctic soils (78). Environmental factors such as pH, vegetation type, and soil temperature can influence methanotroph populations in forest soils (37, 79, 80). In the current study, the relative abundances of the detectable methanotrophs (total, type I, or type II) did not appear to be influenced by pH or by temperature (Table 3). However, they were positively correlated with soil CH_4 concentrations (Table 3) and SWC but negatively correlated with soil O_2 (Table 3). While known bacterial methanotrophs are aerobes, the majority of CH_4 oxidation in riparian-like environments (e.g., rice paddies) occurs at the oxic-anoxic interface in the rhizosphere (81–87). Rahalkar and coworkers reported that no oxygen could be detected in the sediment zone that had the highest abundance of methanotrophs and highest level of methane oxidation activities (88). A major caution in assessing the relative importance of such observations in the context of a forest ecosystem function is that the upland soil clusters α and γ (identified based on distinct *pmoA* clades [89–92]), which are known to be important to CH_4 consumption in forest soils (38, 93, 94), are not represented in this study, since their 16S rRNA gene signatures are not yet known.

For all RZ soils, the abundance of *Crenothrix* was considerable (Fig. 6B). Here, we present it as a methanotroph (95), and as such it represents 68% to ~94% of methanotrophs in these soils. However, this microorganism may be capable of growth on other carbon compounds (95); hence, its role in methane cycling in this particular environment cannot necessarily be assumed. In particular, its potential for utilizing acetate might correlate well with these environments, which presumably favored anaerobic conditions conducive to fermentation, leading to the synthesis of acetate and other organic acids (95).

In conclusion, we characterized the soil microbial community from different positions within a subalpine forested watershed and correlated the microbial communities with historical and contemporary environmental conditions. Our results show that the composition and α- and β-diversity of the microbial communities varied across the three landscape positions tested: HUZ, LUZ, and RZ. SWC, an environmental factor closely related to landscape position within the watershed, appeared to have the highest correlation with the structure of the overall microbial communities as well as the relative abundance of methanotrophs. Methanogens essentially only occurred in riparian soils, while methanotrophs occur in both upland and riparian soils.

ACKNOWLEDGMENTS

We thank Kendra Kaiser, Erin Seybold, Tim Covino, and Liyin Liang for helping collect field environmental data. We also thank the USDA National Forest Service for site access and logistic support.

This project was supported by the U.S. Department of Agriculture (2012-67019-21711) and the National Science Foundation (EPS-1101342 and EAR-1114392).

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the USDA or NSF.

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